

1 **CANCEROUS DISEASE MODIFYING ANTIBODIES**

2 **Reference to Related Applications:**

3 This application is a continuation-in-part of application S.N. 10/348,284, filed January
4 20, 2003, which is a continuation-in-part of application S.N. 09/415,278, filed October 8,
5 1999, now U.S. Patent 6,180,357 B1, the contents of each of which are herein incorporated by
6 reference.

7 **Field of the Invention:**

8 This invention relates to the isolation and production of cancerous disease modifying
9 antibodies (CDMAB) and to the use of these CDMAB in therapeutic and diagnostic processes,
10 optionally in combination with one or more chemotherapeutic agents. The invention further
11 relates to binding assays which utilize the CDMAB of the instant invention.

12 **Background of the Invention:**

13 Each individual who presents with cancer is unique and has a cancer that is as different
14 from other cancers as that person's identity. Despite this, current therapy treats all patients
15 with the same type of cancer, at the same stage, in the same way. At least 30 percent of these
16 patients will fail the first line of therapy, thus leading to further rounds of treatment and the
17 increased probability of treatment failure, metastases, and ultimately, death. A superior
18 approach to treatment would be the customization of therapy for the particular individual. The
19 only current therapy that lends itself to customization is surgery. Chemotherapy and radiation

1 treatment cannot be tailored to the patient, and surgery by itself, in most cases is inadequate for
2 producing cures.

3 With the advent of monoclonal antibodies, the possibility of developing methods for
4 customized therapy became more realistic since each antibody can be directed to a single
5 epitope. Furthermore, it is possible to produce a combination of antibodies that are directed to
6 the constellation of epitopes that uniquely define a particular individual's tumor.

7 Having recognized that a significant difference between cancerous and normal cells is
8 that cancerous cells contain antigens that are specific to transformed cells, the scientific
9 community has long held that monoclonal antibodies can be designed to specifically target
10 transformed cells by binding specifically to these cancer antigens; thus giving rise to the belief
11 that monoclonal antibodies can serve as "Magic Bullets" to eliminate cancer cells.

12 Monoclonal antibodies isolated in accordance with the teachings of the instantly
13 disclosed invention have been shown to modify the cancerous disease process in a manner
14 which is beneficial to the patient, for example by reducing the tumor burden, and will
15 variously be referred to herein as cancerous disease modifying antibodies (CDMAB) or "anti-
16 cancer" antibodies.

17 At the present time, the cancer patient usually has few options of treatment. The
18 regimented approach to cancer therapy has produced improvements in global survival and
19 morbidity rates. However, to the particular individual, these improved statistics do not
20 necessarily correlate with an improvement in their personal situation.

21 Thus, if a methodology was put forth which enabled the practitioner to treat each tumor

1 independently of other patients in the same cohort, this would permit the unique approach of
2 tailoring therapy to just that one person. Such a course of therapy would, ideally, increase the
3 rate of cures, and produce better outcomes, thereby satisfying a long-felt need.

4 Historically, the use of polyclonal antibodies has been used with limited success in the
5 treatment of human cancers. Lymphomas and leukemias have been treated with human
6 plasma, but there were few prolonged remissions or responses. Furthermore, there was a lack
7 of reproducibility and no additional benefit compared to chemotherapy. Solid tumors such as
8 breast cancers, melanomas and renal cell carcinomas have also been treated with human blood,
9 chimpanzee serum, human plasma and horse serum with correspondingly unpredictable and
10 ineffective results.

11 There have been many clinical trials of monoclonal antibodies for solid tumors. In the
12 1980s there were at least 4 clinical trials for human breast cancer which produced only 1
13 responder from at least 47 patients using antibodies against specific antigens or based on tissue
14 selectivity. It was not until 1998 that there was a successful clinical trial using a humanized
15 anti-Her2 antibody in combination with cisplatin. In this trial 37 patients were accessed for
16 responses of which about a quarter had a partial response rate and another half had minor or
17 stable disease progression.

18 The clinical trials investigating colorectal cancer involve antibodies against both
19 glycoprotein and glycolipid targets. Antibodies such as 17-1A, which has some specificity for
20 adenocarcinomas, had undergone Phase 2 clinical trials in over 60 patients with only 1 patient
21 having a partial response. In other trials, use of 17-1A produced only 1 complete response and

1 2 minor responses among 52 patients in protocols using additional cyclophosphamide. Other
2 trials involving 17-1A yielded results that were similar. The use of a humanized murine
3 monoclonal antibody initially approved for imaging also did not produce tumor regression. To
4 date there has not been an antibody that has been effective for colorectal cancer. Likewise
5 there have been equally poor results for lung, brain, ovarian, pancreatic, prostate, and stomach
6 cancers. There has been some limited success in the use of an anti-GD3 monoclonal antibody
7 for melanoma. Thus, it can be seen that despite successful small animal studies that are a
8 prerequisite for human clinical trials, the antibodies that have been tested thus far, have been
9 for the most part, ineffective.

10 Prior Patents:

11 U.S. Patent No. 5,750,102 discloses a process wherein cells from a patient's tumor are
12 transfected with MHC genes which may be cloned from cells or tissue from the patient. These
13 transfected cells are then used to vaccinate the patient.

14 U.S. Patent No. 4,861,581 discloses a process comprising the steps of obtaining
15 monoclonal antibodies that are specific to an internal cellular component of neoplastic and
16 normal cells of the mammal but not to external components, labeling the monoclonal antibody,
17 contacting the labeled antibody with tissue of a mammal that has received therapy to kill
18 neoplastic cells, and determining the effectiveness of therapy by measuring the binding of the
19 labeled antibody to the internal cellular component of the degenerating neoplastic cells. In
20 preparing antibodies directed to human intracellular antigens, the patentee recognizes that
21 malignant cells represent a convenient source of such antigens.

1 U.S. Patent No. 5,171,665 provides a novel antibody and method for its production.
2 Specifically, the patent teaches formation of a monoclonal antibody which has the property of
3 binding strongly to a protein antigen associated with human tumors, e.g. those of the colon and
4 lung, while binding to normal cells to a much lesser degree.

5 U.S. Patent No. 5,484,596 provides a method of cancer therapy comprising surgically
6 removing tumor tissue from a human cancer patient, treating the tumor tissue to obtain tumor
7 cells, irradiating the tumor cells to be viable but non-tumorigenic, and using these cells to
8 prepare a vaccine for the patient capable of inhibiting recurrence of the primary tumor while
9 simultaneously inhibiting metastases. The patent teaches the development of monoclonal
10 antibodies which are reactive with surface antigens of tumor cells. As set forth at col. 4, lines
11 45 et seq., the patentees utilize autochthonous tumor cells in the development of monoclonal
12 antibodies expressing active specific immunotherapy in human neoplasia.

13 U.S. Patent No. 5,693,763 teaches a glycoprotein antigen characteristic of human
14 carcinomas is not dependent upon the epithelial tissue of origin.

15 U.S. Patent No. 5,783,186 is drawn to anti-Her2 antibodies which induce apoptosis in
16 Her2 expressing cells, hybridoma cell lines producing the antibodies, methods of treating
17 cancer using the antibodies and pharmaceutical compositions including said antibodies.

18 U.S. Patent No. 5,849,876 describes new hybridoma cell lines for the production of
19 monoclonal antibodies to mucin antigens purified from tumor and non-tumor tissue sources.

20 U.S. Patent No. 5,869,268 is drawn to a method for generating a human lymphocyte
21 producing an antibody specific to a desired antigen, a method for producing a monoclonal

1 antibody, as well as monoclonal antibodies produced by the method. The patent is particularly
2 drawn to the production of an anti-HD human monoclonal antibody useful for the diagnosis
3 and treatment of cancers.

4 U.S. Patent No. 5,869,045 relates to antibodies, antibody fragments, antibody
5 conjugates and single chain immunotoxins reactive with human carcinoma cells. The
6 mechanism by which these antibodies function is two-fold, in that the molecules are reactive
7 with cell membrane antigens present on the surface of human carcinomas, and further in that
8 the antibodies have the ability to internalize within the carcinoma cells, subsequent to binding,
9 making them especially useful for forming antibody-drug and antibody-toxin conjugates. In
10 their unmodified form the antibodies also manifest cytotoxic properties at specific
11 concentrations.

12 U.S. Patent No. 5,780,033 discloses the use of autoantibodies for tumor therapy and
13 prophylaxis. However, this antibody is an anti-nuclear autoantibody from an aged mammal. In
14 this case, the autoantibody is said to be one type of natural antibody found in the immune
15 system. Because the autoantibody comes from "an aged mammal", there is no requirement that
16 the autoantibody actually comes from the patient being treated. In addition the patent discloses
17 natural and monoclonal anti-nuclear autoantibody from an aged mammal, and a hybridoma cell
18 line producing a monoclonal anti-nuclear autoantibody.

19 Summary of the Invention:

20 The instant inventors have previously been awarded U.S. Patent 6,180,357, entitled
21 "Individualized Patient Specific Anti-Cancer Antibodies" directed to a process for selecting

1 individually customized anti-cancer antibodies which are useful in treating a cancerous
2 disease. For the purpose of this document, the terms "antibody" and "monoclonal antibody"
3 (mAb) may be used interchangeably and refer to intact immunoglobulins produced by
4 hybridomas, immunoconjugates and, as appropriate, immunoglobulin fragments and
5 recombinant proteins derived from immunoglobulins, such as chimeric and humanized
6 immunoglobulins, F(ab') and F(ab')₂ fragments, single-chain antibodies, recombinant
7 immunoglobulin variable regions (Fv)s etc. Furthermore, it is within the purview of this
8 invention to conjugate standard chemotherapeutic modalities, e.g. radionuclides, with the
9 CDMAB of the instant invention, thereby focusing the use of said chemotherapeutics. The
10 CDMAB can also be conjugated to toxins, cytotoxic moieties or enzymes e.g. biotin
11 conjugated enzymes.

12 The prospect of individualized anti-cancer treatment will bring about a change in the
13 way a patient is managed. A likely clinical scenario is that a tumor sample is obtained at the
14 time of presentation, and banked. From this sample, the tumor can be typed from a panel of
15 pre-existing cancerous disease modifying antibodies. The patient will be conventionally
16 staged but the available antibodies can be of use in further staging the patient. The patient can
17 be treated immediately with the existing antibodies and/or a panel of antibodies specific to the
18 tumor can be produced either using the methods outlined herein or through the use of phage
19 display libraries in conjunction with the screening methods herein disclosed. All the
20 antibodies generated will be added to the library of anti-cancer antibodies since there is a
21 possibility that other tumors can bear some of the same epitopes as the one that is being
22 treated. The antibodies produced according to this method may be useful to treat cancerous

1 disease in any number of patients who have cancers that bind to these antibodies.

2 Using substantially the process of US 6,180,370, the mouse monoclonal antibodies
3 7BD-33-11A and 1A245.6 were obtained following immunization of mice with cells from a
4 patient's breast tumor biopsy. Within the context of this application, anti-cancer antibodies
5 having either cell-killing (cytotoxic) or cell-growth inhibiting (cytostatic) properties will
6 hereafter be referred to as cytotoxic. These antibodies can be used in aid of staging and
7 diagnosis of a cancer, and can be used to treat tumor metastases. The 7BD-33-11A and
8 1A245.6 antigen was expressed on the cell surface of a broad range of human cell lines from
9 different tissue origins. The breast cancer cell line MCF-7 and prostate cancer cell line PC-3
10 were the only 2 cancer cell lines tested that were susceptible to the cytotoxic effects of either
11 7BD-33-11A or 1A245.6.

12 The result of 7BD-33-11A and 1A245.6 cytotoxicity against breast and prostate
13 cancer cells in culture was further extended by establishing its anti-tumor activity *in vivo*. In
14 an *in vivo* model of human cancer, the MB-231 breast cancer cells or PC-3 prostate cancer
15 cells were implanted underneath the skin at the scruff of the neck of severe combined
16 immunodeficient (SCID) mice, as they are incapable of rejecting the human tumor cells
17 due to a lack of certain immune cells. Pre-clinical xenograft tumor models are considered
18 valid predictors of therapeutic efficacy. Xenografts in mice grow as solid tumors
19 developing stroma, central necrosis and neo-vasculature in the same manner as naturally
20 occurring cancers. The mammary tumor cell line MB-231 and the prostate tumor cell line
21 PC-3 have been evaluated as an *in vivo* xenograft model in immunodeficient mice. The
22 successful engraftment or 'take-rate' of both the MB-231 and PC-3 tumors and the

Deleted:

1 sensitivity of the tumors to standard chemotherapeutic agents have characterized them as
2 suitable models. The MB-231 parental cell line and variants of the cell line and the PC-3
3 androgen-independent cell line have been used successfully in xenograft tumor models to
4 evaluate a wide range of therapeutic agents that used as clinical chemotherapeutic agents.

5 As outlined and described in S.N. 10/348,284, 7BD-33-11A and 1A245.6 prevented
6 tumor growth and reduced tumor burden in a preventative *in vivo* model of human breast
7 cancer. Monitoring continued past 150 days post-treatment. 7BD-33-11A never developed
8 tumors and 87.5 percent of the 7BD-33-11A treatment group was still alive at over 6 months
9 post-implantation. Conversely, the isotype control group had 100 percent mortality by day 72
10 (23 days post-treatment). 1A245.6 treated mice reached 100 percent mortality by day 151
11 post-treatment, which is greater than 6 times longer than the isotype control treatment group.
12 Therefore 1A245.6, and to a greater extent 7BD-33-11A, enhanced survival and decreased the
13 tumor burden in a breast cancer model.

14 Also as outlined and described in S.N. 10/348,284, both 7BD-33-11A and 1A245.6
15 significantly suppressed tumor growth and decreased tumor burden in an established *in vivo*
16 model of human breast cancer. By day 80 (23 days post-treatment), 7BD-33-11A treated mice
17 had 83 percent lower mean tumor volumes in comparison to isotype control group ($p=.001$).
18 1A245.6 treatment also produced lower mean tumor volumes on this day, 35 percent ($p=.135$).
19 Using survival as a measure of antibody efficacy, it was estimated that the risk of dying in the
20 7BD-33-11A treatment group was about 16 percent of the isotype control group ($p=0.0006$) at
21 around 60 days post-treatment. 100 percent of the isotype control group died by 50 days post-
22 treatment. In comparison, 1A245.6 treated mice survived until 100 days post-treatment and

1 60% of the 7BD-33-11A treatment groups were still alive at 130 days post-treatment. This
2 data demonstrate that both 1A245.6 and 7BD-33-11A treatments conferred a survival and
3 reduced tumor burden benefit compared to the control treated group. 7BD-33-11A and
4 1A245.6 treatment appeared safe, as it did not induce any signs of toxicity, including reduced
5 body weight and clinical distress. Thus, 7BD-33-11A and 1A245.6 treatment was efficacious
6 as it both delayed tumor growth and enhanced survival compared to the control-treated groups
7 in a well-established model of human breast cancer.

8 In addition to the beneficial effects in the established *in vivo* tumor model of breast
9 cancer, 7BD-33-11A and 1A245.6 treatment also had anti-tumor activity against PC-3 cells in
10 a preventative *in vivo* prostate cancer model. In this prostate xenograft model, 7BD-33-11A
11 and 1A245.6 were given separately to mice 1 day prior to implantation of tumor cells followed
12 by weekly injections for 7 weeks. 7BD-33-11A and 1A245.6 treatment was significantly
13 ($p=.001$ and $.017$ respectively) more effective in suppressing tumor growth shortly after the
14 treatment period than an isotype control antibody. At the end of the treatment phase, mice
15 given 7BD-33-11A or 1A245.6 had tumors that grew to only 31 and 50 percent of the isotype
16 control group respectively.

17 For PC-3 SCID xenograft models, body weight can be used as a surrogate indicator of
18 disease progression. On day 52, both 7BD-33-11A and 1A245.6 treatment significantly
19 ($p=.002$ and $.004$ respectively) prevented the loss of body weight by 54 and 25 percent
20 respectively in comparison to isotype control. Mice were monitored for survival post-
21 treatment. At 11 days post-treatment, isotype and buffer control mice had reached 100 percent
22 mortality. Conversely, 7BD-33-11A and 1A245.6 reached 100 percent mortality at day 38

1 post-treatment, 3 times longer than the control groups. Thus, 7BD-33-11A and 1A245.6
2 treatment was efficacious as it both delayed tumor growth, prevented body weight loss and
3 extended survival compared to the isotype control treated group in a well-established model of
4 human prostate cancer.

5 In addition to the preventative *in vivo* tumor model of prostate cancer, 7BD-33-11A
6 demonstrated anti-tumor activity against PC-3 cells in an established *in vivo* tumor model. In
7 this xenograft model, PC-3 prostate cancer cells were transplanted subcutaneously into SCID
8 mice such that the tumor reached a certain size before antibody treatment. Treatment with
9 7BD-33-11A was again compared to isotype control. It was shown that the 7BD-33-11A
10 treatment group had significantly ($p < 0.024$) smaller mean tumor volumes compared with the
11 isotype control treated group immediately following treatment. 7BD-33-11A treatment
12 mediated tumor suppression by 36 percent compared to the isotype control group. The anti-
13 tumor activities of 7BD-33-11A, in several different cancer models, make it an attractive anti-
14 cancer therapeutic agent.

15 The binding of 7BD-33-11A and 1A245.6 towards normal human tissues was
16 determined. By IHC staining, the majority of the tissues failed to express the 7BD-33-11A
17 antigen, including the vital organs, such as the kidney, heart, and lung. 7BD-33-11A stained
18 the salivary gland, liver, pancreas, stomach, prostate and duodenum, and strongly stained the
19 tonsil. Results from tissue staining indicated that 7BD-33-11A showed restricted binding to
20 various cell types but had binding to infiltrating macrophages, lymphocytes, and fibroblasts.
21 For 1A245.6, a wider range of tissues was positively stained. For the majority of cases,
22 staining was restricted to the epithelium or infiltrating macrophages, lymphocytes, and

1 fibroblasts. However, positive staining was seen on both cardiac muscle and hepatocytes.
2 7BD-33-11A and 1A245.6 displayed both membrane and cytoplasmic staining patterns.

3 Localization of the 7BD-33-11A and 1A245.6 antigen and its prevalence within breast
4 cancer patients is important in assessing the benefits of immunotherapy to. To address antigen
5 expression in breast tumors from cancer patients, tumor tissue samples from 50 individual
6 breast cancer patients were screened for expression of either the 7BD-33-11A or 1A245.6
7 antigen. The results of the study showed that 36 percent of tissue samples positively stained
8 for the 7BD-33-11A antigen. Expression of 7BD-33-11A within patient samples appeared
9 specific for cancer cells as staining was restricted to malignant cells. In addition, 7BD-33-11A
10 stained 0 of 10 samples of normal tissue from breast cancer patients. On the other hand,
11 1A245.6 stained 98 percent of breast cancer tissue samples. 1A245.6 also stained 8 out of 10
12 samples of normal tissue from breast cancer patients. However, in general this staining was
13 much weaker than that observed with the breast cancer tissue samples and was generally
14 restricted to infiltrating fibroblasts. 7BD-33-11A and 1A245.6 expression was further
15 evaluated based on breast tumor expression of the receptors for the hormones estrogen and
16 progesterone, which play an important role in the development, treatment, and prognosis of
17 breast tumors. No correlation was apparent between expression of the 1A245.6 antigen and
18 expression of the receptors for either estrogen or progesterone. There was a slight correlation
19 between estrogen or progesterone receptor expression and expression of 7BD-33-11A; tissues
20 with receptor expression had slightly higher 7BD-33-11A expression. When tumors were
21 analyzed based on their stage, or degree to which the cancer advanced, results suggested a
22 trend towards greater positive expression with higher tumor stage for 7BD-33-11A and higher

intensity staining with higher tumor stage for 1A245.6. However, the results were limited by the small sample size.

To further extend the potential therapeutic benefit of 7BD-33-11A and 1A245.6, the frequency and localization of the antigen within various human cancer tissues was determined. Several cancer types, in addition to breast cancer, expressed the 7BD-33-11A antigen. The positive human cancer types included skin (1/2), lung (3/4), liver (2/3), stomach (4/5), thyroid (2/2), prostate (1/1), uterus (4/4) and kidney (3/3). Some cancers did not express the antigen; these included ovary (0/3), testis (0/1), brain (0/2) and lymph node (0/2). For 1A245.6, as with the normal human tissue array, a multitude of cancers from various human tissue types were positively stained. Greater staining was seen on malignant cells of the skin, lung, liver, uterus, kidney, stomach and bladder. As with human breast cancer tissue, localization of 7BD-33-11A and 1A245.6 occurred both on the membrane and within the cytoplasm of these tumor cells. So, in addition to the 7BD-33-11A and 1A245.6 antibody binding to cancer cell lines *in vitro*, there is evidence that the antigen is expressed in humans, and on multiple types of cancers.

In toto, this data demonstrates that both the 7BD-33-11A and 1A245.6 antigen is a cancer associated antigen and is expressed in humans, and is a pathologically relevant cancer target. Further, this data also demonstrates the binding of 7BD-33-11A and 1A245.6 antibody to human cancer tissues, and can be used appropriately for assays that can be diagnostic, predictive of therapy, or prognostic. In addition, the cell membrane localization of this antigen permits the use of this antigen, its gene or derivatives, its protein or its variants to be used for assays that can be diagnostic, predictive of therapy, or prognostic.

1 In all, this invention teaches the use of the 7BD-33-11A or 1A245.6 antigen as a target
2 for a therapeutic agent, that when administered can reduce the tumor burden of a cancer
3 expressing the antigen in a mammal, and can also lead to a prolonged survival of the treated
4 mammal. This invention also teaches the use of CDMAB (7BD-33-11A/1A245.6), and its
5 derivatives, to target its antigen to reduce the tumor burden of a cancer expressing the antigen
6 in a mammal, and to prolong the survival of a mammal bearing tumors that express this
7 antigen. Furthermore, this invention also teaches the use of detecting the 7BD-33-11A or
8 1A245.6 antigen in cancerous cells that can be useful for the diagnosis, prediction of therapy,
9 and prognosis of mammals bearing tumors that express this antigen.

10 If a patient is refractory to the initial course of therapy or metastases develop, the
11 process of generating specific antibodies to the tumor can be repeated for re-treatment.
12 Furthermore, the anti-cancer antibodies can be conjugated to red blood cells obtained from that
13 patient and re-infused for treatment of metastases. There have been few effective treatments
14 for metastatic cancer and metastases usually portend a poor outcome resulting in death.
15 However, metastatic cancers are usually well vascularized and the delivery of anti-cancer
16 antibodies by red blood cells can have the effect of concentrating the antibodies at the site of
17 the tumor. Even prior to metastases, most cancer cells are dependent on the host's blood
18 supply for their survival and an anti-cancer antibody conjugated to red blood cells can be
19 effective against *in situ* tumors as well. Alternatively, the antibodies may be conjugated to
20 other hematogenous cells, e.g. lymphocytes, macrophages, monocytes, natural killer cells, etc.

21 There are five classes of antibodies and each is associated with a function that is
22 conferred by its heavy chain. It is generally thought that cancer cell killing by naked

antibodies are mediated either through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). For example murine IgM and IgG2a antibodies can activate human complement by binding the C-1 component of the complement system thereby activating the classical pathway of complement activation which can lead to tumor lysis. For human antibodies, the most effective complement activating antibodies are generally IgM and IgG1. Murine antibodies of the IgG2a and IgG3 isotype are effective at recruiting cytotoxic cells that have Fc receptors which will lead to cell killing by monocytes, macrophages, granulocytes and certain lymphocytes. Human antibodies of both the IgG1 and IgG3 isotype mediate ADCC.

Another possible mechanism of antibody mediated cancer killing may be through the use of antibodies that function to catalyze the hydrolysis of various chemical bonds in the cell membrane and its associated glycoproteins or glycolipids, so-called catalytic antibodies.

There are two additional mechanisms of antibody mediated cancer cell killing which are more widely accepted. The first is the use of antibodies as a vaccine to induce the body to produce an immune response against the putative antigen that resides on the cancer cell. The second is the use of antibodies to target growth receptors and interfere with their function or to down regulate that receptor so that effectively its function is lost.

Accordingly, it is an objective of the invention to utilize a method for producing CDMAB from cells derived from a particular individual which are cytotoxic with respect to cancer cells while simultaneously being relatively non-toxic to non-cancerous cells, in order to isolate hybridoma cell lines and the corresponding isolated monoclonal antibodies and antigen

1 binding fragments thereof for which said hybridoma cell lines are encoded.

2 It is an additional objective of the invention to teach CDMAB and antigen binding
3 fragments thereof.

4 It is a further objective of the instant invention to produce CDMAB whose cytotoxicity
5 is mediated through ADCC.

6 It is yet an additional objective of the instant invention to produce CDMAB whose
7 cytotoxicity is mediated through CDC.

8 It is still a further objective of the instant invention to produce CDMAB whose
9 cytotoxicity is a function of their ability to catalyze hydrolysis of cellular chemical bonds.

10 A still further objective of the instant invention is to produce CDMAB which are useful
11 in a binding assay for the diagnosis, prognosis, and monitoring of cancer.

12 Other objects and advantages of this invention will become apparent from the
13 following description wherein are set forth, by way of illustration and example, certain
14 embodiments of this invention.

15 Brief Description of the Figures:

16 The patent or application file contains at least one drawing executed in color. Copies
17 of this patent or patent application publication with color drawing(s) will be provided by the
18 Office upon request and payment of the necessary fee.

19 Figure 1. Survival of tumor-bearing mice after treatment with 7BD-33-11A, 1A245.6 or
20 isotype control antibody in a preventative MB-231 xenograft study. Mice were monitored for

1 survival for 200 days post-treatment.

2 Figure 2. Effect of 7BD-33-11A and 1A245.6 on tumor growth in a preventative MB-231
3 breast cancer model. The dashed line indicates the period during which the antibody was
4 administered. Data points represent the mean \pm SEM.

5 Figure 3. Survival of tumor-bearing mice after treatment with 7BD-33-11A, 1A245.6 or
6 isotype control antibody in an established MB-231 xenograft study. Mice were monitored for
7 survival for 130 days post-treatment.

8 Figure 4. Effect of 7BD-33-11A and 1A245.6 on tumor growth in a preventative PC-3
9 prostate cancer model. The dashed line indicates the period during which the antibody was
10 administered. Data points represent the mean \pm SEM.

11 Figure 5. Histogram showing mean body weight of the different treatment groups over the
12 duration of the preventative PC-3 xenograft study. Data are presented as the mean \pm SEM for
13 each group at each time point.

14 Figure 6. Survival of tumor-bearing mice after treatment with 7BD-33-11A, 1A245.6, isotype
15 or buffer control antibody in a preventative PC-3 xenograft study. Mice were monitored for
16 survival for 38 days post-treatment.

17 Figure 7. Effect of 7BD-33-11A and 1A245.6 on tumor growth in an established PC-3
18 prostate cancer model. The dashed line indicates the period during which the antibody was
19 administered. Data points represent the mean \pm SEM.

20 Figure 8. Histogram showing mean body weight of the different treatment groups over the
21 duration of the established PC-3 xenograft study. Data are presented as the mean \pm SEM for
22 each group at each time point.

1 Figure 9. Normal Human Brain A. 7BD-33-11A. B. 1A245.6. C. Negative isotype control.
2 Magnification is 200X.
3 Figure 10. Normal Human Heart A. 7BD-33-11A. B. 1A245.6 (arrows indicate positive
4 staining). C. Negative isotype control. Magnification is 200X.
5 Figure 11. Normal Human Stomach Antrum. A. 7BD-33-11A (arrows indicate positive
6 staining of gastric gland epithelium). B. 1A245.6 (arrows indicate positive staining of gastric
7 gland epithelium). C. Negative isotype control. Magnification is 200X.
8 Figure 12. Representative micrograph of 7BD-33-11A binding to human breast cancer tumor
9 (infiltrating duct carcinoma; Panel A; black arrows: sheets of tumor cells, yellow arrow: tumor
10 stroma) and human normal breast (Panel B). Magnification is 200X.
11 Figure 13. Representative micrograph of 1A245.6 binding to human breast cancer tumor
12 (infiltrating duct carcinoma; Panel A; black arrows: sheets of tumor cells, yellow arrow: tumor
13 stroma) and human normal breast (Panel B; black arrows: fibroblasts). Magnification is 200X.
14 Figure 14. Renal Cell Carcinoma. A. 7BD-33-11A (arrows indicate positive staining in sheets
15 of tumor cells). B. 1A245.6 (arrows indicate positive staining in sheets of tumor cells). C.
16 Negative isotype control. Magnification is 200X.

17

18 Detailed Description Of The Invention:

19 Example 1

20 The hybridoma cell lines 7BD-33-11A and 1A245.6 were deposited, in accordance
21 with the Budapest Treaty, with the American Type Culture Collection, 10801 University Blvd.,

1 Manassas, VA 20110-2209 on January 8, 2003, under Accession Number PTA-4890 and PTA-
2 4889 respectively. In accordance with 37 CFR 1.808, the depositors assure that all restrictions
3 imposed on the availability to the public of the deposited materials will be irrevocably
4 removed upon the granting of a patent.

5 7BD-33-11A and 1A245.6 monoclonal antibody was produced by culturing the
6 hybridomas in CL-1000 flasks (BD Biosciences, Oakville, ON) with collections and reseeded
7 occurring twice/week and with purification according to standard antibody purification
8 procedures with Protein G Sepharose 4 Fast Flow (Amersham Biosciences, Baie d'Urfé, QC).

9

10 In Vivo MB-231 Preventative Survival Tumor Experiments

11 As outlined in S.N. 10/348,284, and with reference to Figure 1, 4 to 8 week old female
12 SCID mice were implanted with 5 million MB-231 human breast cancer cells in 100
13 microlitres saline injected subcutaneously in the scruff of the neck. The mice were randomly
14 divided into 3 treatment groups of 10. On the day prior to implantation, 20 mg/kg of either
15 7BD-33-11A, 1A245.6 test antibodies or isotype control antibody (known not to bind MB-231
16 or PC-3 cells) was administered intraperitoneally at a volume of 300 microliters after dilution
17 from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM KH₂PO₄, 137
18 mM NaCl and 20 mM Na₂HPO₄. The antibodies were then administered once per week for a
19 period of 7 weeks in the same fashion. Tumor growth was measured about every seventh day
20 with calipers for up to 10 weeks or until individual animals reached the Canadian Council for
21 Animal Care (CCAC) end-points. Body weights of the animals were recorded for the duration

1 of the study. At the end of the study all animals were euthanised according to CCAC
2 guidelines.

3 In continuation from S.N. 10/348,284, there was a post-treatment survival benefit
4 (Figure 1) associated with treatment with either 7BD-33-11A or 1A245.6. 7BD-33-11A never
5 developed tumors and only 1 mouse had died by day 200 (151 days post-treatment). In
6 contrast, all of the isotype control mice had died by day 23 post-treatment. The 1A245.6
7 treated group did not reach 100 percent mortality until day 151 post-treatment which is greater
8 than 6 times longer than the isotype control treatment group. In summary 1A245.6 and 7BD-
9 33-11A increased survival and decreased tumor burden in a breast tumor model of human
10 cancer.

11 Example 2

12 *In Vivo* MB-231 Established Tumor Experiments

13 As outlined in S.N. 10/348,284, and with reference to Figures 2 and 3, 5 to 6 week old
14 female SCID mice were implanted with 5 million MB-231 human breast cancer cells in 100
15 microlitres saline injected subcutaneously in the scruff of the neck. Tumor growth was
16 measured with calipers every week. When the majority of the cohort reached a tumor volume
17 of 100 mm³ (range 50-200 mm³) at 34 days post-implantation 8-10 mice were randomly
18 assigned into each of 3 treatment groups. 7BD-33-11A, 1A245.6 test antibodies or isotype
19 control antibody was administered intraperitoneally with 15 mg/kg of antibodies at a volume
20 of 150 microliters after dilution from the stock concentration with a diluent that contained 2.7
21 mM KCl, 1 mM KH₂PO₄, 137 mM NaCl and 20 mM Na₂HPO₄. The antibodies were then
22

1 administered 3 times per week for 10 doses in total in the same fashion until day 56 post-
2 implantation. Tumor growth was measured about every seventh day with calipers until day 59
3 post-implantation or until individual animals reached the CCAC end-points. Body weights of
4 the animals were recorded for the duration of the study. At the end of the study all animals
5 were euthanised according to CCAC guidelines.

6 In continuation from S.N. 10/348,284, there was a post-treatment tumor burden
7 reduction (Figure 2) and survival benefit (Figure 3) associated with treatment with either 7BD-
8 33-11A or 1A245.6. At day 80 (23 days post-treatment) both 7BD-33-11A and 1A245.6 had
9 decreased mean tumor volumes compared to isotype control treatment; 83 ($p=.001$) and 35
10 percent ($p=.135$) respectively. A *Cox proportional model* was used to compare the hazard
11 (risk) rates in the different groups. In this method, the hazard rate of every group is compared
12 with the hazard of the isotype control group. At approximately 60 days post-treatment, the risk
13 of dying in the 7BD-33-11A group was 16 percent in comparison to the isotype control
14 treatment group ($p=.0006$). The survival benefit associated with 7BD-33-11A appeared to
15 continue on well past the 100 day post-treatment mark. At day 130 post-treatment, 7BD-33-
16 11A had 60% survival while all of the isotype control mice had died at day 50 post-treatment.
17 1A245.6 had double the survival time in comparison to the isotype control: 100 versus 50 days
18 post-treatment. Therefore both 7BD-33-11A and 1A245.6 lowered the tumor burden and
19 increased survival in comparison to a control antibody in a well recognized model of human
20 breast cancer disease suggesting pharmacologic and pharmaceutical benefits of these
21 antibodies for therapy in other mammals, including man.

22

1 Example 3

2 *In Vivo* PC-3 Preventative Tumor Experiments

3 With reference to the data shown in Figures 4 and 5, 4 to 8 week old, male SCID mice
4 were implanted with 1 million PC-3 human prostate cancer cells in 100 microliters saline
5 injected subcutaneously in the scruff of the neck. The mice were randomly divided into 4
6 treatment groups of 8. On the day prior to implantation 20 mg/kg of 7BD-33-11A or 1A245.6
7 test antibody or isotype control antibody or buffer control was administered intraperitoneally at
8 a volume of 300 microliters after dilution from the stock concentration with a diluent that
9 contained 2.7 mM KCl, 1 mM KH₂PO₄, 137 mM NaCl and 20 mM Na₂HPO₄. The antibodies
10 and buffer control were then administered once per week for a period of 7 weeks in the same
11 fashion. Tumor growth was measured about every 7th day with calipers for up to 10 weeks or
12 until individual animals reached the CCAC end-points or day 52. Body weights of the animals
13 were recorded for the duration of the study. At the end of the study all animals were euthanised
14 according to CCAC guidelines.

15 Using the least significant difference method (LSD) to specify the different groups, it is
16 apparent that both 7BD-33-11A and 1A245.6 significantly reduced the tumor burden in treated
17 mice in comparison to controls (Figure 4). After treatment (day 52), 7BD-33-11A prevented
18 tumor growth by 69 percent (p=.001) in comparison to isotype control and 1A245.6 also
19 prevented tumor growth by 50 percent (p=.017) in comparison to isotype control. Similar
20 findings were found when comparisons were made to the buffer control. In a PC-3 prostate
21 cancer xenograft model, body weight can be used as a surrogate indicator of disease
22 progression (Figure 5). A repeated analysis of variance (Rep. ANOVA) indicates there was no

1 significant difference in body weight between the isotype and buffer control group. Analysis
2 of variance determined that at day 52, 7BD-33-11A had a significantly higher body weight
3 than both of the control groups and the 1A245.6 treated group ($p<.03$). Overall, 7BD-33-11A
4 prevented body weight loss by 54 percent ($p=.002$) while 1A245.6 prevented body weight loss
5 by 25 percent ($p=.004$) compared to the isotype control group. Mice were monitored post-
6 treatment for survival (Figure 6). 100 percent of 7BD-33-11A and 1A245.6 treated mice
7 reached mortality by day 38 post-treatment, which is greater than 3 times longer than the
8 isotype and buffer control treatment group, 11 days post-treatment.

9 In summary, 7BD-33-11A and 1A245.6 antibody treatment reduced tumor burden,
10 delayed disease progression and extended survival in comparison to an isotype control
11 antibody and a buffer control in a well-recognized model of human prostate cancer. These
12 results suggest a potential pharmacologic and pharmaceutical benefit of these antibodies (7BD-
13 33-11A and 1A245.6) as a therapy beyond breast cancer.

14 15 Example 4

16 *In Vivo* PC-3 Established Tumor Experiments:

17 Male SCID mice, 4 to 8 weeks old, were implanted with 1 million PC-3 prostate cancer
18 cells in 100 microliters saline injected subcutaneously in the scruff of the neck. Tumor growth
19 was measured with calipers every week. When the majority of the cohort reached a tumor
20 volume of 275 mm³ (range 144-406 mm³) at 21 days post implantation, 9-10 mice were
21 randomized into each of 4 treatment groups. 7BD-33-11A or 1A245.6 or isotype control
22 antibody was administered intraperitoneally with 20 mg/kg/dose at a volume of 300 microliters

1 after dilution from the stock concentration with a diluent that contained 2.7 mM KCl, 1 mM
2 KH_2PO_4 , 137 mM NaCl and 20 mM Na_2HPO_4 . The antibodies were then administered 3 times
3 per week for a total of 10 doses in the same fashion until day 43 post-implantation. Tumor
4 growth was measured about every seventh day with calipers for the duration of the study or
5 until individual animals reached CCAC end-points. Body weights of the animals were
6 recorded for the duration of the study. At the end of the study all animals were euthanised
7 according to CCAC guidelines.

8 At the time of randomization the mean tumor volumes and the standard deviations in
9 each group were similar. Statistically there was no difference in body weight between the
10 groups. This indicated that true randomization had occurred. As shown in Figure 7, the
11 antibody 7BD-33-11A was able to significantly suppress tumor growth by 36 percent ($p=.024$)
12 in comparison to isotype control at the end of the 3-week treatment period. 1A245.6 showed
13 no significant difference when compared to isotype or buffer control treatment groups.
14 Likewise, neither 7BD-33-11A or 1A245.6 showed any significant difference in comparison to
15 isotype or buffer control treatment groups in terms of body weight (Figure 8). All groups
16 displayed the same significant amount of body weight loss throughout the study ($p<.001$).

17 In summary, 7BD-33-11A is significantly more effective than the isotype control
18 antibody in suppressing tumor growth in an established tumor xenograft model of prostate
19 cancer in SCID mice. Therefore treatment with 7BD-33-11A significantly decreased the tumor
20 burden of established tumors in two well-recognized models of human cancer disease (breast
21 and prostate) suggesting pharmacologic and pharmaceutical benefits of this antibody for
22 therapy in other mammals, including man.

1

2 Example 5

3 Normal Human Tissue Staining

4 IHC studies were conducted to characterize the 7BD-33-11A and 1A245.6 antigen
5 distribution in humans. IHC optimization studies were performed previously in order to
6 determine the conditions for further experiments. 7BD-33-11A and 1A245.6 monoclonal
7 antibody was produced and purified as stated above.

8 Tissue sections were deparaffinized by drying in an oven at 58°C for 1 hour and
9 dewaxed by immersing in xylene 5 times for 4 minutes each in Coplin jars. Following
10 treatment through a series of graded ethanol washes (100%-75%) the sections were re-hydrated
11 in water. The slides were immersed in 10 mM citrate buffer at pH 6 (Dako, Toronto, Ontario)
12 then microwaved at high, medium, and low power settings for 5 minutes each and finally
13 immersed in cold PBS. Slides were then immersed in 3% hydrogen peroxide solution for 6
14 minutes, washed with PBS three times for 5 minutes each, dried, incubated with Universal
15 blocking solution (Dako, Toronto, Ontario) for 5 minutes at room temperature. 7BD-33-11A,
16 1A245.6, monoclonal mouse anti-vimentin (Dako, Toronto, Ontario) or isotype control
17 antibody (directed towards *Aspergillus niger* glucose oxidase, an enzyme which is neither
18 present nor inducible in mammalian tissues; Dako, Toronto, Ontario) were diluted in antibody
19 dilution buffer (Dako, Toronto, Ontario) to its working concentration (5µg/mL for each
20 antibody) and incubated overnight for 1 hour at room temperature. The slides were washed
21 with PBS 3 times for 5 minutes each. Immunoreactivity of the primary antibodies was
22 detected/visualized with HRP conjugated secondary antibodies as supplied (Dako Envision

1 System, Toronto, Ontario) for 30 minutes at room temperature. Following this step the slides
2 were washed with PBS 3 times for 5 minutes each and a color reaction developed by adding
3 DAB (3,3'-diaminobenzidine tetrahydrachloride, Dako, Toronto, Ontario) chromogen
4 substrate solution for immunoperoxidase staining for 10 minutes at room temperature.
5 Washing the slides in tap water terminated the chromogenic reaction. Following
6 counterstaining with Meyer's Hematoxylin (Sigma Diagnostics, Oakville, ON), the slides were
7 dehydrated with graded ethanols (75-100%) and cleared with xylene. Using mounting media
8 (Dako Faramount, Toronto, Ontario) the slides were coverslipped. Slides were microscopically
9 examined using an Axiovert 200 (Zeiss Canada, Toronto, ON) and digital images acquired and
10 stored using Northern Eclipse Imaging Software (Mississauga, ON). Results were read, scored
11 and interpreted by a pathologist.

12 Binding of antibodies to 59 normal human tissues was performed using a human,
13 normal organ tissue array (Imgenex, San Diego, CA). Table 1 presents a summary of the
14 results of 7BD-33-11A and 1A245.6 staining of an array of normal human tissues. From the
15 table, there are 3 categories of tissue staining. A group of tissues was completely negative.
16 These tissues included normal skin, brain (Figure 9A), ovary, thymus, thyroid, small bowel,
17 esophagus, heart (Figure 10A), gall bladder and lymph node for 7BD-33-11A. For 1A245.6,
18 the completely negative tissues comprised of skin, sub-cutis fat, esophagus and brain (Figure
19 9B). A second group of tissues comprised tissues that demonstrated positive staining. These
20 included the liver and pancreas for 7BD-33-11A. The tonsil had the strongest staining with
21 this antibody. For 1A245.6, positive staining occurred in the liver, heart (Figure 10B), testis,
22 thyroid, adrenal gland and myometrium. As with 7BD-33-11A, 1A245.6 stained the tonsil the

1 strongest. A third group of tissues included tissues in which staining was positive in the tissue
2 section, but was limited to infiltrating macrophages, lymphocytes, fibroblasts or the
3 epithelium, for example the stomach for both 7BD-33-11A and 1A245.6 (Figure 11A and B
4 respectively). It should be noted that the 7BD-33-11A antigen is not present on cells of
5 several of the vital organs, including kidney, heart (Figure 10A) and lung. Overall, 7BD-33-
6 11A binds to a smaller subset of normal human tissues compared to 1A245.6 with weak to
7 moderate binding in the tissues that are positive. 1A245.6 staining, albeit more extensive, is
8 also generally weak to moderate in intensity and in the majority of cases is limited to the
9 epithelium of the stained tissue. These results suggest that the antigen for 7BD-33-11A is not
10 widely expressed on normal tissues, and that the antibody would bind specifically to a limited
11 number of tissues in humans. In addition, the antigen for 1A245.6, besides being present in
12 the heart and liver, is limited to epithelium and infiltrating lymphocytes, macrophages and
13 fibroblasts.

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Table 1: IHC On Normal Human Tissue

Sec. No.	Organ	IA245.6	7BD33-11A	Vimentin
1	*Skin	-	-	+++ Fibroblasts
2	*Skin	-	-	+++ Fibroblasts
3	Subcutis fat	-	-	++ Adipocytes
4	Breast	+/- Fibroblasts	-	++ Endothelium, Smooth muscles of blood vessels
5	Breast	+++ Lobular epithelium	+/- Fibroblasts	++ Blood vessels, stroma
6	Spleen	++ Lymphocytes	+/- Lymphocytes	+++ Sinusoidal endothelium, Lymphocytes
7	Spleen	+++ Lymphocytes	+/- Lymphocytes	+++ Sinusoidal endothelium, Lymphocytes
8	Lymph node	++ Endothelium of blood vessels, Lymphocytes	-	+++ Lymphocytes
9	Lymph node	+ Endothelium of blood vessels	-	++ Blood vessels, Lymphocytes
10	Skeletal muscle	+/- Endothelium of blood vessels	-	+/- Blood vessels
11	Nasal mucosa	- NR	- NR	- NR
12	Lung	+/- Interstitial cells (macrophages)	-	++ Alveolar epithelium, Macrophages
13	Lung	+/- Bronchiolar epithelium, ++ Macrophages	+ Macrophages	++ Alveolar epithelium, Macrophages, Lymphocytes
14	Bronchus	- NR	- NR	++ Chondrocytes, NR
15	Heart	++ Cardiac muscle	++	++ Blood vessels
16	Salivary gland	++ Acinar epithelium	+ Acinar epithelium	+++ Blood vessels, Peripheral nerves
17	Liver	+++ Hepatocytes	++ Hepatocytes	++ Blood vessels
18	Liver	+++ Hepatocytes	++ Hepatocytes	+++ Blood vessels, Macrophages
19	Liver	+ Hepatocytes	+/- Hepatocytes	+/- Blood vessels
20	Old Haskler	++ Mucosal epithelium, Fibroblasts, Smooth muscle fibers	-	+++ Lymphocytes
21	Pancreas	+++ Acinar epithelium, Islets of Langerhans	+ Acinar epithelium	+++ Acinar epithelium, Blood vessels
22	Pancreas	+++ Acinar epithelium, Islets of Langerhans	++ Acinar epithelium, Islets of Langerhans	++ Acinar epithelium, Blood vessels
23	Tortal	+++ Keratin, + Lymphocytes	+++ Keratin, +/- Lymphocytes	+++ Lymphocytes
24	Esophagus	-	-	++ Blood vessels
25	Esophagus	-	-	+++ Blood vessels
26	***Stomach body	++ Gastric gland epithelium, ++ Lymphocytes in lamina propria	++ Gastric gland epithelium	+++ Blood vessels & Fibroblasts
27	***Stomach body	+++ Gastric gland epithelium, + Lymphocytes in lamina propria	++ Gastric gland epithelium	+++ Lymphocytes, Blood vessels, Fibroblasts
28	Stomach antrum	+/- Gastric gland epithelium, + Lymphocytes	++ Gastric gland epithelium	+++ Lymphocytes
29	Stomach, Smooth muscle	-	-	+++ Lymphocytes, Blood vessels
30	Duodenum	+++ Lymphocytes	+ Intestinal gland epithelium	++ Fibroblasts, Blood vessels
31	Small bowel	+/- Lymphocytes in lamina propria	-	+ Lymphocytes, Blood vessels
32	Small bowel	+/- Lymphocytes in lamina propria	-	+++ Lymphocytes in lamina propria
33	Appendix	+++ Mucosal epithelium, Lymphocytes	++ Lymphocytes	+++ Lymphocytes, Blood vessels
34	Colon	+/- Lymphocytes	+/- Macrophages in lamina propria	++ Lymphocytes
35	Colon	+ Lymphocytes	-	++ Lymphocytes, Blood vessels
36	Rectum	+/- Lymphocytes, Blood vessels	-	++ Lymphocytes, Blood vessels
37	Kidney cortex	++ Tubular epithelium	-	+++ Glomerular capillary, Blood vessels
38	Kidney cortex	+++ Tubular epithelium	+ Tubular epithelium	+++ Tubular epithelium, glomerular capillary, Blood vessels
39	Kidney medulla	++ Tubular epithelium	-	++ Renal tubule epithelium, Lipocytes, Fibroblasts
40	Urinary bladder	++ Transitional epithelium	+/- Transitional epithelium	++ Blood vessels
41	Prostate	+++ Glandular epithelium	++ Glandular epithelium	+++ Glandular epithelium, Blood vessels
42	Prostate	+++ Glandular epithelium	++ Glandular epithelium	+++ Glandular epithelium, Blood vessels
43	Seminal vesicle	+ Mucosal epithelium	+/- Mucosal epithelium	+/-
44	Testis	++ Germinal epithelium, + Leydig cells	+/- Leydig cells	+++ Germinal epithelium
45	Endometrium proliferative	+++ Glandular epithelium, + Stroma	-	+++ Endometrial glands, Stroma
46	Endometrium secretory	+++ Glandular epithelium, Stroma	-	++ Glandular epithelium, +++ Blood vessels
47	Myometrium	+ Smooth muscle fibers	+/- Fibroblasts	++ Smooth muscle fibers, Blood vessels
48	Uterine cervix	+/- Fibroblasts	-	+++ Fibroblasts
49	Salpinx	+ Mucosal epithelium, Blood vessels	-	+/- Mucosal epithelium, +++ Blood vessels
50	***Ovary	+ Stromal cells	-	+++ Stromal cells
51	Placenta, villi	+ Trophoblasts	-	++ Blood vessels
52	Placenta, villi	++ Trophoblasts	-	++ Blood vessels
53	Umbilical cord	-	-	++ Fibroblasts
54	Adrenal gland	++ Endocrine cells	++ +/-	++ +/-
55	Thyroid	+/- Follicular cells	-	++ Follicular cells, Blood vessels
56	Thymus	+ Lymphocytes	-	+++ Lymphocytes
57	Brain white matter	-	-	++ Astrocytes
58	Brain gray matter	-	-	++ Blood vessels
59	Cerebellum	-	-	++ Cerebellar cortex

7 Abbreviations: * : Originally pigmented stratum basale; ** : Endogenous cytoplasmic pigment/back ground
8 staining; *** : Stomach antrum (not stomach body); **** : Ovarian stroma only; NR : The section is not
9 representative; CS : The section is completely sloughed.

Example 6

Human Tumor Tissue Staining

An IHC study was undertaken to determine the cancer association of the 7BD-33-11A and 1A245.6 antigen with human breast cancers and whether either antibody was likely to recognize human cancers. A comparison was made for vimentin (positive control), and an antibody directed towards *Aspergillus niger* glucose oxidase, an enzyme which is neither present nor inducible in mammalian tissues (negative control). A breast cancer tissue array derived from 50 breast cancer patients and 10 samples derived from non-neoplastic breast tissue in breast cancer patients were used (Imgenex Corporation, San Diego, CA). The following information was provided for each patient: age, sex, and diagnosis. The procedure for IHC from Example 5 was followed. All antibodies were used at a working concentration of 5 µg/ml.

Table 2 provides a binding summary of 7BD-33-11A and 1A245.6 antibody staining of a breast cancer tissue array. Each array contained tumor samples from 50 individual patients. Overall, 36 percent of the 50 patients tested were positive for the 7BD-33-11A antigen (Figure 12A) compared to 98 percent for 1A245.6 (Figure 13A). For 7BD-33-11A, 0 out of 10 normal breast tissue samples from breast cancer patients were positive (Figure 12B). Conversely, 9 out of 10 normal breast tissue samples were positive for 1A245.6. However, staining was due to infiltrating fibroblasts in the majority of cases (Figure 13B). No correlation between estrogen and progesterone receptor status was evident for 1A245.6 (Table

1 3). There were a slightly higher number of positive 7BD-33-11A antigen tissues that were also
2 estrogen and progesterone receptor expressers (Table 4). For the 7BD-33-11A antigen, it also
3 appeared there was a trend to greater positive expression with higher tumor stage (Table 4) and
4 for 1A245.6, the intensity of tissue staining appeared to correlate with higher tumor stage
5 (Table 3). Both the 7BD-33-11A and 1A245.6 staining was specific for cancerous cells and
6 staining occurred on both the membrane and within the cytoplasm. The staining pattern, from
7 both 7BD-33-11A and 1A245.6, showed that in patient samples, the antibody is highly specific
8 for malignant cells and the respective antigens are present on the cell membrane thereby
9 making it an attractive druggable target.

1 Table 2- IHC On Human Breast Tumor Tissue

Sec. No.	Age	Sex	Diagnosis	1A245.6	7BD-33-11A
1	F	28	Infiltrating duct carcinoma	+++ MC	++ MC
2	F	71	Solid papillary carcinoma	+++ MC	+/-
3	F	26	Infiltrating duct carcinoma	++ MC	-
4	F	43	Infiltrating duct carcinoma	++ MC	+/-
5	F	39	Infiltrating duct carcinoma	+ MC Tumor, +++ Necrotic area	+/-
6	F	46	Ductal carcinoma <i>in situ</i>	++ MC	+/-
7	F	47	Infiltrating duct carcinoma	+++ MC Tumor, ++ stroma	+ MC
8	M	67	Infiltrating duct carcinoma	+++ MC	+ MC
9	F	33	Infiltrating duct carcinoma	++ MC	-
10	F	47	Infiltrating duct carcinoma	++ MC	-
11	F	49	Invasive lobular carcinoma	- Tumor, +/- Fibroblasts	-
12	F	46	Infiltrating duct carcinoma	+++ MC	-
13	F	39	Infiltrating duct carcinoma	++ MC	-
14	F	43	Infiltrating lobular carcinoma	+++ MC	+/-
15	F	54	Infiltrating lobular carcinoma	++ MC	+/-
16	F	58	Infiltrating duct carcinoma	++ MC Tumor, Stroma, +++ Necrotic area	+/-
17	F	37	Infiltrating duct carcinoma	+++ MC	-
18	F	43	Infiltrating duct carcinoma	+++ MC Tumor, Stroma	+++ M/C
19	F	51	Infiltrating duct carcinoma	+++ MC	+ MC
20	F	80	Medullary carcinoma	++ MC	-
21	F	36	Infiltrating duct carcinoma	++ MC Tumor, Stroma	-
22	F	59	Infiltrating duct carcinoma	+ MC	+/- Blood vessels
23	F	34	Ductal carcinoma <i>in situ</i>	++ MC Tumor, Stroma, +++ Necrotic area	+Tumor, ++ Necrotic area
24	F	54	Infiltrating duct carcinoma	++ MC	+/-
25	F	47	Infiltrating duct carcinoma	+++ MC	++ MC
26	F	53	Infiltrating duct carcinoma	++ MC	-
27	F	59	Infiltrating duct carcinoma	+ MC Tumor, Stroma, Endothelium of blood vessels	+/- Necrotic area
28	F	60	Signet ring cell carcinoma	+++ MC	-
29	F	37	Infiltrating duct carcinoma	+++ MC	++ MC
30	F	46	Infiltrating duct carcinoma	++ MC	+/-
31	F	35	Infiltrating duct carcinoma	+/-	-
32	F	47	Infiltrating duct carcinoma	+Tumor, +++ Necrotic area	-
33	F	54	Infiltrating duct carcinoma	++ MC	-
34	F	47	Infiltrating duct carcinoma	+ MC Tumor, Stroma	-
35	F	41	Infiltrating duct carcinoma	+++ MC	-
36	F	38	Infiltrating duct carcinoma	+++ MC	-
37	F	55	Infiltrating duct carcinoma	+ MC Tumor, Stroma	-
38	F	65	Infiltrating duct carcinoma	++ MC Tumor, Stroma	-
39	M	66	Infiltrating duct carcinoma	+ MC Tumor, Stroma	-
40	F	44	Infiltrating duct carcinoma	++ MC	-
41	F	52	Metastatic carcinoma in lymph node	++ MC	-
42	F	32	Metastatic carcinoma in lymph node	++ MC	-
43	F	58	Metastatic carcinoma in lymph node	+++ MC	+/-
44	F	52	Metastatic carcinoma in lymph node	++ MC	-
45	F	58	Metastatic carcinoma in lymph node	+ MC	-
46	F	38	Metastatic carcinoma in lymph node	+++ MC	-
47	F	45	Metastatic carcinoma in lymph node	+/-	-
48	F	45	Metastatic carcinoma in lymph node	++ MC	-
49	F	29	Metastatic carcinoma in lymph node	++ MC	-
50	F	61	Metastatic carcinoma in lymph node	++ MC	-
*51	F	46	Nipple	++ Sebaceous glands	-
*52	F	47	Nipple	++ MC	-
*53	F	40	Normal Breast	-	-
*54	F	43	Normal Breast	+/- Fibroblasts	-
*55	F	40	Normal Breast	++ Lobular epithelium, Fibroblasts, Endothelium	-
*56	F	40	Normal Breast	+/- Fibroblasts	-
*57	F	45	Normal Breast	+/- Fibroblasts	-
*58	F	44	Normal Breast	+/- Fibroblasts	-
*59	F	37	Normal Breast	++ Lobular epithelium, Fibroblasts	-
*60	F	51	Normal Breast	+/- Fibroblasts	-

2 Abbreviations: PS : the section is partially detached; * : Non-neoplastic breast tissue in breast cancer patient.

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1 Table 3: IHC Correlation Summary For 1A245.6

		Binding Score					Total positive	% positive of total
		Total #	-	+/-	+	++		
Patient Samples	Tumor	50	1	2	8	23	16	49
	Normal	10	1	5 (Fibroblasts)	0	4	0	9
ER Status	ER+	28	0	1	2	14	11	28
	ER-	22	1	1	6	9	5	21
	Unknown	0	0	0	0	0	0	0
PR Status	PR+	19	0	0	1	8	10	19
	PR-	30	1	2	7	14	6	29
	Unknown	1	0	0	0	0	0	1
AJCCTumorStage	T1	4	0	1	1	1	1	4
	T2	21	1	0	6	9	5	20
	T3	20	0	1	1	10	8	20
	T4	5	0	0	0	3	2	5

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3 Table 4: IHC Correlation Summary For 7BD-33-11A

		Binding Score					Total positive	% positive of total
		Total #	-	+/-	+	++		
Patient Samples	Tumor	50	30	12	4	3	1	20
	Normal	10	10	0	0	0	0	0
ER Status	ER+	28	16	9	1	2	0	12
	ER-	22	15	3	2	1	1	7
	Unknown	0	0	0	0	0	0	0
PR Status	PR+	19	9	6	2	2	0	10
	PR-	30	20	6	2	1	1	10
	Unknown	1	1	0	0	0	0	0
AJCC Tumor Stage	T1	4	4	0	0	0	0	0
	T2	21	14	3	2	1	1	7
	T3	20	11	6	2	1	0	9
	T4	5	1	3	0	1	0	4

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5 To determine whether either the 7BD-33-11A or 1A245.6 antigen is expressed on other
6 human cancer tissues in addition to breast cancer, both antibodies were individually tested on a
7 multiple human tumor tissue array (Imgenex, San Diego, CA). The following information was
8 provided for each patient: age, sex, organ and diagnosis. The staining procedure used was the
9 same as the one outlined in Example 5. Vimentin was used as a positive control antibody and
10 the same negative control antibody was used as described for the human breast tumor tissue
11 array. All antibodies were used at a working concentration of 5 µg/mL.

12 As outlined in Table 5, 7BD-33-11A stained a number of various human cancers
13 besides breast. The following tumor types were positive for 7BD-33-11A: skin (1/2), lung
14 (3/4), liver (2/3), stomach (4/5), thyroid (2/2), prostate (1/1), uterus (4/4) and kidney (3/3)

1 (Figure 14A). Several other tumor types also occasionally stained positive. Other tumor
2 tissues were negative for 7BD-33-11A expression; ovary (0/3), testis (0/1), brain (0/2) and
3 lymph node (0/2). Conversely, 1A245.6 stained every tumor tissue type tested. However,
4 some of the strongest staining was seen on malignant cells of the skin, lung, liver, uterus,
5 kidney (Figure 14B), stomach and bladder. As seen with the breast cancers, 7BD-33-11A and
6 1A245.6 staining was localized on the membrane and within the cytoplasm of cancerous cells.

7 Therefore, it appears that the 7BD-33-11A and 1A245.6 antigen is not solely found on
8 the membranes of breast cancers but also on the membrane of a large variety of tumor types
9 including prostate. These results indicate that both 7BD-33-11A and 1A245.6 have potential
10 as a therapeutic drug in a wide variety of tumor types in addition to breast and prostate cancer.

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Table 5: IHC On Human Multi-Tumor Tissue Array

Sec. No.	Age	Sex	Organ	Diagnosis	1A245.6	7BD-33-11A
1	59	M	Skin	Malignant melanoma	++ M/C	++ M/C
2	25	F	Skin	SSC	++ M/C	-
3	50	F	Breast	Infiltrating ductal carcinoma	++ M/C	++ M/C
4	57	F	Breast	Invasive papillary carcinoma	+ M/C	-
5	35	F	Breast	Infiltrating lobular carcinoma	+ M/C	F
6	40	M	Lymph node	Malignant lymphoma, immunoplastic	+ M/C	-
7	58	M	Lymph node	Metastatic adenocarcinoma from stomach	+ M/C	-
8	53	F	Bone	Osteosarcoma	++ M/C	++ M/C
9	26	M	Bone	Giant cell tumor	++ M/C	-
10	40	M	Bone	Chondrosarcoma	-	CS
11	51	F	Soft tissue	Liposarcoma	+/-	-
12	47	F	Soft tissue	Neurofibromatosis	+/-	-
13	74	M	Nasal cavity	Inverted papilloma	+ M/C	+/-
14	57	M	Larynx	SCC	+ Tumor, ++ Lymphocytes, Stroma	+/-
15	60	M	Lung	Adenocarcinoma	+++ M/C	++ M/C
16	51	F	Lung	SCC	++ M/C	+ M/C
17	68	F	Lung	Adenocarcinoma	+ M/C	-
18	60	M	Lung	Small cell carcinoma	++ M/C	+ M/C
19	88	F	Tongue	SCC	+ M/C	+/-
20	34	F	Parotid gland	Pleomorphic adenoma	+/- mucin	-
21	50	F	Parotid gland	Warthin tumor	+++ M/C	+++ M/C
22	40	F	Parotid gland	Pleomorphic adenoma	+ M/C	+/-
23	56	M	Submandibular gland	Salivary duct carcinoma	+ M/C	-
24	69	F	Liver	Cholangiocarcinoma	+++ M/C	++ M/C
25	51	M	Liver	Metastatic gastric Ca.	++ M/C	-
26	64	M	Liver	HCC	+++ M/C	++ M/C
27	62	F	Gallbladder	Adenocarcinoma	++ M/C	+ M/C
28	64	F	Pancreas	Adenocarcinoma	++ M/C	++ M/C
29	68	M	Esophagus	SCC	+ M/C	+/-
30	73	M	Stomach	Adenocarcinoma (poorly differentiated)	++ M/C	+ M/C
31	63	M	Stomach	Adenocarcinoma (moderately differentiated)	++ M/C	+/-
32	59	F	Stomach	Signet ring cell carcinoma	++ M/C	+/-
33	62	M	Stomach	Malignant lymphoma	+ M/C	+ Blood vessels
34	51	M	Stomach	Borderline stromal tumor	+++ M/C	+ M/C
35	42	M	Small intestine	Malignant stromal tumor	++ M/C	-
36	52	F	Appendix	Pseudomyxoma peritonii	- PS	-
37	53	M	Colon	Adenocarcinoma	+ M/C	+/-
38	67	M	Rectum	Adenocarcinoma	++ M/C	-
39	75	F	Kidney	Transitional cell carcinoma	+++ M/C	++ M/C
40	54	F	Kidney	Renal cell carcinoma	++ M/C	+/-
41	75	F	Kidney	Renal cell carcinoma	+++ M/C	++ M/C
42	65	M	Urinary bladder	Carcinoma (poorly differentiated)	++ M/C	-
43	67	M	Urinary bladder	Transitional cell carcinoma (high grade)	+++ M/C	+++ M/C
44	62	M	Prostate	Adenocarcinoma	+ M/C	+/-
45	30	M	Testis	Seminoma	+ M/C	-
46	68	F	Uterus	Endometrial adenocarcinoma	+++ M/C	++ M/C
47	57	F	Uterus	Leiomyosarcoma	+ C	+/-
48	45	F	Uterus	Leiomyoma	+ C	+/-
49	63	F	Uterine cervix	SCC	+ Tumor, ++ Stroma, Lymphocytes	+/-
50	12	F	Ovary	Endodermal sinus tumor	++ M/C	-
51	33	F	Ovary	Mucinous adenocarcinoma	+ M/C	-
52	70	F	Ovary	Fibrosarcoma	++ M/C	-
53	67	F	Adrenal gland	Cortical carcinoma	++ M/C	+ M/C
54	61	F	Adrenal gland	Pheochromocytoma	+++ M/C	-
55	54	M	Thyroid	Papillary carcinoma	+++ M/C	++ M/C
56	58	F	Thyroid	Minimally invasive follicular carcinoma	++ M/C	+ M/C
57	74	M	Thymus	Thymoma	+ C	+/-
58	66	F	Brain	Meningioma	++ M/C	-
59	62	M	Brain	Glioblastoma multiforme	+ M/C	-

2 Abbreviations: M: Membrane staining; C: Cytoplasmic staining; M/C: Membrane-cytoplasmic staining; CS: the
3 section is completely sloughed; PS: the section is partially sloughed; F: The section is folded; SSC: Squamous cell
4 carcinoma; HCC: Hepatocellular carcinoma.

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1 All patents and publications mentioned in this specification are indicative of the levels
2 of those skilled in the art to which the invention pertains. All patents and publications are
3 herein incorporated by reference to the same extent as if each individual publication was
4 specifically and individually indicated to be incorporated by reference.

5 It is to be understood that while a certain form of the invention is illustrated, it is not to
6 be limited to the specific form or arrangement of parts herein described and shown. It will be
7 apparent to those skilled in the art that various changes may be made without departing from
8 the scope of the invention and the invention is not to be considered limited to what is shown
9 and described in the specification. One skilled in the art will readily appreciate that the
10 present invention is well adapted to carry out the objects and obtain the ends and advantages
11 mentioned, as well as those inherent therein. Any oligonucleotides, peptides, polypeptides,
12 biologically related compounds, methods, procedures and techniques described herein are
13 presently representative of the preferred embodiments, are intended to be exemplary and are
14 not intended as limitations on the scope. Changes therein and other uses will occur to those
15 skilled in the art which are encompassed within the spirit of the invention and are defined by
16 the scope of the appended claims. Although the invention has been described in connection
17 with specific preferred embodiments, it should be understood that the invention as claimed
18 should not be unduly limited to such specific embodiments. Indeed, various modifications of
19 the described modes for carrying out the invention which are obvious to those skilled in the art
20 are intended to be within the scope of the following claims.

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